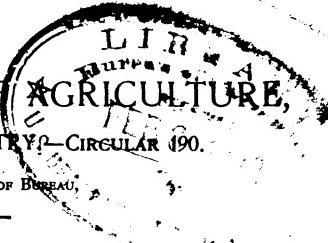


U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—CIRCULAR 190.

A. D. MELVIN, CHIEF OF BUREAU,



# THE VACCINATION OF CATTLE AGAINST TUBERCULOSIS.

BY

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*Of the Experiment Station,*

AND

JOHN R. MOHLER and HENRY J. WASHBURN,  
*Of the Pathological Division.*

[Redacted from the Twenty-seventh Annual Report of the Bureau of Animal Industry (1910).]



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1912.

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### INTRODUCTION.

The heavy annual losses which are caused by the ravages of tuberculosis among domesticated animals have been appreciated by the inhabitants of infected countries for many years. Owing to this realization of the extent of the havoc wrought by this insidious disease, earnest thought and study have been devoted by scientific forces in all civilized countries to the question of its eradication.

It was known from the first that the fight against tuberculosis among cattle would be a prolonged one because of the hidden manner in which the disease makes its attack, but when the suggestion was made that cattle might be safely and completely immunized against the disease the advantages which might arise from this method of procedure became at once apparent. It is obvious that if the young animals of an infected herd or locality can be thoroughly protected from tubercular infection the root of the matter has been reached, and it is then only a question of time when all the remaining animals can be disposed of and the premises can be cleaned and disinfected and kept free from tuberculosis.

An appreciation of the advantages accruing from immunization in our tuberculous herds led the Bureau of Animal Industry to inaugurate the tests here recorded. It was clearly seen that it was most desirable to devise some method whereby cattle could be immunized rapidly and without danger to themselves or their attendants. Therefore a number of the most promising methods of applying the immunizing agent have been tried, and while none of them have proved perfect, some have been more or less efficacious in enhancing the powers of resistance of the animals treated.

### REVIEW OF RECENT LITERATURE.

At the Ninth International Congress of Veterinary Medicine, held at The Hague in September, 1909, the subject of immunization against tuberculosis received most interested attention, and the papers

which dealt with this question were actively discussed. Among the opinions which were expressed during this discussion we quote from Dr. A. Eber, of Leipsic; Dr. J. F. Heymanns, of Ghent; Dr. Klimmer, of Dresden; Dr. Vallée, of Alfort; and Dr. Arloing, of Lyon.

Dr. Eber stated that the following conclusions had been reached by him after careful study of the entire question and after observing the results of numerous preventive inoculations:

The receptivity of young cattle to experimental infection by virulent tubercle bacilli may be materially diminished by previous inoculation with the Koch bacillus, even of varied origin and virulence.

The immunity thus conferred is not absolute. The immunized cattle will succumb from the effects of a sufficiently heavy dose of tuberculous virus.

The increase of resistance is not complete for some time (at least three months) after inoculation, and has entirely disappeared at the end of the first or second year.

The degree and duration of the experimental immunity are influenced by the individual resistance, and up to a certain point by the quality of the vaccine used.

No experimental method permits one to foresee the manner in which the vaccinated animals will comport themselves toward natural or enzootic contagion. Practice alone must decide the value of immunization in the struggle against bovine tuberculosis. Tuberculin does not positively disclose the existence of tuberculous centers upon animals previously treated by injections of living tubercle bacilli.

Science has not yet granted us a method of inoculation which permits effective struggle against tuberculosis in regions seriously infected.

The new researches simply shed a light upon preventive inoculation when combined with other prophylactic measures (killing animals affected with open tuberculosis, raising the calves on sterilized milk, plowing pastures) during the struggle against tuberculosis.

In practice one prefers methods of immunization which permit annual re-inoculation. Nevertheless, more exact researches must be instituted for the purpose of determining if annual preventive inoculation is sufficient in every case to confer satisfactory immunity.

It is of great importance for the future to study further the influence of the mode of inoculation (intravenous, subcutaneous, digestive) upon the quality and duration of the immunity acquired, keeping constantly in mind at the same time the various doors of entrance of natural infection (digestive or respiratory).

Dr. Heymanns limited his report to a discussion of the method which he discovered, and which consists in inserting into the animal vaccinated the unattenuated tubercle bacilli inclosed within a dialyzing membrane.

Healthy animals thus vaccinated offer greater resistance to infection, whether by inoculation or stabbing, than the checks. Although the duration and the degree of immunity are limited, the results obtained during four years upon more than 10,000 subjects have been most encouraging.

In practicing annually tuberculination and vaccination of all the cattle in contaminated stables without separating the tuberculous

from the nontuberculous and without heating the milk, 86 per cent of the tuberculous centers have been practically wiped out after three or four years without the appearance of any serious trouble during the experiment. Upon stables more seriously contaminated Heymanns recognizes the fact that simple vaccination and tuberculination are ineffective, because the healthy cattle reinfect themselves in proportion to the intensity of the contagion. Upon such farms, in addition to vaccination, appropriate prophylaxis must be imposed.

The tuberculous cattle vaccinated and tested with tuberculin annually react less and less to the tuberculin, and by the third application of the tuberculin test 50 to 60 per cent of them fail to show any thermic elevation whatever.

The results of more than 1,000 autopsies upon vaccinated cattle indicate that in general healthy cattle that have been vaccinated have remained immune to tuberculosis and that the tuberculous animals, having ceased to react to tuberculin, present an arrested tuberculosis, even a regression, but the total absence of tuberculous lesions is exceptional.

In conclusion, Heymanns states that his antituberculous vaccination is a method practical and efficacious and, taken together with prophylactic measures, forms a valuable base in the struggle against tuberculosis, permitting one to struggle victoriously against this foe of our stables and this danger of infection to man.

Dr. Klimmer has examined the following methods of vaccination: First, the bovo-vaccination of Von Behring; second, the vaccination with "tauruman" of Koch and Schütz; third, the method of Heymanns; and fourth, the method of Klimmer.

The method of Klimmer consists in vaccinating with attenuated human tubercle bacilli. The nontuberculous animals are vaccinated twice during the first year, and those that are tuberculous are vaccinated every three months. The following year all the animals are vaccinated but once.

The preventive methods of Von Behring and of Koch and Schütz have proven practically inefficient. The reason is found in the short duration of the immunity which they produce and in the impossibility, while following sanitary regulations, of revaccinating either with bovo-vaccine or the tauruman.

Klimmer claims that it is not possible at this date to estimate the curative value of the method devised by Heymanns.

The method of Klimmer has not yet been sufficiently tested. Nevertheless, out of 43 vaccinated animals which had been exposed, some of them during many years, to natural tuberculous contagion, not a single one had developed tuberculosis up to the time of his report. Tuberculous young cattle have been vaccinated and from one to three years later have been killed, when autopsy has shown

that the progress of the disease has been arrested, the tuberculous centers have become encapsulated and frequently calcified, and that there has been no formation of new tuberculous centers.

Dr. Vallée believes that we must recognize that, in spite of the enormous benefits realized on all sides, none of the proposed methods of vaccination has yet furnished definite results. He states:

I. Nevertheless, the inoculation of cattle by any method with virulent human tubercle bacilli confers an appreciable resistance against various methods of experimental infection and also against natural contagion.

II. The resistance conferred is directly proportional to the quantity and virulence of the bacilli injected, but however great their value, the immunity conferred by them does not persist longer than 12 to 18 months.

III. The introduction of living bacilli as a vaccine contaminates the entire organism. This peculiarity necessitates a special guard over the animals immunized, should they be sent to slaughter during the six months which follow the last vaccination.

IV. Whatever the mode chosen for introducing the vaccine, the resistance conferred is insufficient to assure the complete resorption of the bacilli inoculated for prolonging immunity.

V. The resistance to infection by the digestive tube of the animal vaccinated by that method is incomparably superior to that acquired by animals by the intravenous method, because it permits the organism to obtain complete resorption of the virulent material inoculated. Considering the frequency of infection through the digestive canal in cattle, the application of vaccinating material through the mouth appears preferable to every other method.

VI. Vaccination by the digestive method can not be made entirely free from danger of infection if one uses virulent bacilli of the bovine type. The use of bacilli of the human type of slight virulence is, therefore, preferable, as these furnish results comparatively equal to those of the bovine type.

VII. Vaccination by way of the mouth is not easily obtained except upon very young subjects.

VIII. Vaccination by way of the mouth does not place the animals entirely beyond danger of infection with tuberculosis. It permits them to resist for more than a year contact with cattle which present open lesions of tuberculosis and, following this, present no lesions beyond insignificant tubercular nodules in the various glands. On this account it merits systematic study and further practical application.

IX. The resistance conferred by subcutaneous methods is inferior to that obtained by way of the circulation.

X. Vaccination by the use of killed bacilli has given results inferior to those obtained with living and virulent organisms.

XI. No definite conclusion can yet be actually formulated in regard to the various methods of immunization under discussion, but their systematic application will permit a determination of their real practical value.

The method of immunization proposed by Arloing does not exactly resemble any of the other systems which are at present in use. Most of these latter methods depend on the employment of attenuated tubercle bacilli, but the vaccines employed by Arloing are not composed of bacilli modified specially in any case either by heat or anti-septics or by a passage through the organisms of cold-blooded or

other animals. On the contrary, his vaccine contains living bacilli of bovine origin profoundly modified in their tubercle-producing power by a long series of cultures in the depth of glycerinated bouillon. The modifications which they have taken on are henceforth fixed and of such a kind that these bacilli form races indefinitely transmissible. These races, comparable to the antianthrax vaccines of Pasteur, can no longer cause tuberculosis of the viscera and glands under the conditions where they are recommended to be employed. Being without danger to the monkey, Arloing considers that they are also without danger to man. By their characters these vaccines are somewhat similar to the virulent vaccines of Prof. Klimmer, of Dresden. They can not cause any fatal infection in the ox, which is contrary to the bovo-vaccine of Von Behring and the tauruman of Koch and Schütz, since these may be fatal to from 7 to 8 out of every 1,000 subjects vaccinated.

Arloing concludes by stating that the phase of experimental researches in tuberculosis vaccination is not closed, and it is to be hoped that by perseverance in laboratory studies the methods will be perfected, and we will know better the conditions which follow and those which guarantee success. But such as they are to-day, it would be negligent not to profit by the results acquired to try and restrain the ravages of bovine tuberculosis by associating vaccination with ordinary prophylactic measures, as one does for other contagious maladies.

As a result of these various papers and the discussions which they elicited, the following resolutions were adopted by the Ninth International Congress held at The Hague in 1909:

1. At the present time there is no vaccination which in itself is sufficient to combat in an efficient manner bovine tuberculosis in heavily infected herds.
2. In how far it is possible to bring about a more successful issue of the difficult struggle against bovine tuberculosis by a combination of vaccination with prophylactic and hygienic measures must be demonstrated by new practical experiments.
3. The congress urgently requests the Governments to grant the means for extensive experiments to examine the methods of vaccination against bovine tuberculosis under the different conditions of agricultural practice.

#### THE BUREAU EXPERIMENTS.

Probably no methods for the immunization of cattle against tuberculosis have been more widely discussed or have given better results than those known as Pearson's and Von Behring's. The two are practically alike, and consist of the intravenous injection of living cultures of human tubercle bacilli of a virulence too low to cause a progressive tuberculosis in cattle. The tubercle bacilli are grown in artificial cultures, and, in a very finely subdivided condition, suspended in fluid in definitely known quantites, are injected into a vein of the animal to be protected. The methods of bovo-vaccination of

Pearson and Von Behring were both tested, with what results will follow later.

In addition to studying these methods, attempts were made to cause immunity by subcutaneous injections of tubercle bacilli of different degrees of virulence as well as by the transfusion of blood from artificially immunized to susceptible animals.

Finally, a test was made of what is known as Heymann's capsule method of protective treatment, for the efficiency of which the discoverer, after applying it to a large number of animals in his own country, makes very strong claims.

#### TEST ACCORDING TO VON BEHRING'S METHOD.

On June 2, 1906, the first nine calves and on June 12 the tenth calf in the following list were given each an intrajugular injection of 3 cubic centimeters of a suspension of tubercle bacilli of the human type. The tubercle bacilli used to make the suspension were from what was known as "Case 30," and each cubic centimeter of the suspension was equal to 0.0013 gram of tubercle bacilli:

- Bull calf No. 427, about 2 to 2½ months old.
- Bull calf No. 429, about 2 to 2½ months old.
- Bull calf No. 431, about 2 to 2½ months old.
- Heifer calf No. 432, about 2½ to 3 months old.
- Heifer calf No. 435, about 2 to 2½ months old.
- Heifer calf No. 436, about 2 to 2½ months old.
- Bull calf No. 438, about 2 to 2½ months old.
- Bull calf No. 439, about 2½ to 3 months old.
- Bull calf No. 441, about 2 to 2½ months old.
- Bull calf No. 444, about 3 to 3½ months old.

The ages of the calves as above given refer to the date on which the injections were made.

With the exception of a subsequent brief elevation of temperature and the development of a small tumor in the skin over the jugular at the point of injection in several of the calves, the injections were followed by no marked adverse conditions.

On September 7, 1906, each of the 10 calves was given a second intrajugular injection of tubercle bacilli prepared from culture "Case 30." The suspension used for the second injection, of which the dose was 3 c. c., represented 0.02 gram of tubercle bacilli per cubic centimeter.

The second injection was followed by a more marked elevation of temperature than the first, which may, in part at least, be attributed to the mechanically irritant action of the injected tubercle bacilli in the fine pulmonary capillaries.

Of the 10 calves, 1 (No. 444) was killed and examined post-mortem without being exposed to tuberculosis to determine what changes had

been caused by the injections of tubercle bacilli it had received. The remaining 9 were exposed to a tuberculous environment, as follows:

- Nos. 427 and 432, October 30, 1906.
- Nos. 429 and 435, February 27, 1907.
- Nos. 431 and 436, April 25, 1907.
- Nos. 438 and 441, July 25, 1907.
- No. 439, November 8, 1907.

Control animals were added at the time of each exposure, but as a matter of convenience the controls will be treated separately, as such treatment will facilitate a better comparison between the several protective methods against tuberculosis that were tested.

The tuberculous environment to which the animals used in these experiments were exposed was a large cow stable with an adjacent cow yard. In this stable and yard the infection was provided by a number of cattle affected with advanced tuberculosis, of which it was definitely known that they were freely expelling tubercle bacilli. In the yard into which the cattle were turned daily for from 2 to 8 hours, depending upon the state of the weather, all the cattle treated, checks and diseased, were allowed to mingle as they chose; in the stable the cattle were made to occupy stalls in such rotation that the exposure of the different individuals was equalized as much as possible.

The dates on which the 10 calves were killed and a short memorandum of the lesions found on autopsy follow:

No. 444, treated but not exposed, reacted to tuberculin October 17, 1907 (13 months after last bovo-vaccination injection). Killed November 7, 1907. No lesions found, but firm pulmonary adhesions and one small glistening nodule, 3 mm. in diameter, in the lung. No tubercle bacilli on microscopic examination or animal inoculation were found in the minute glistening nodule, which may have been a small healed tubercle caused by bacilli from one of the intravenous injections.

No. 427, killed April 23, 1908, after having been exposed to a tuberculous environment for about 18 months. No lesions of tuberculosis.

No. 432, killed March 9, 1909, after having been exposed to a tuberculous environment over 2 years. No lesions of tuberculosis.

No. 429, killed July 16, 1909, after having been exposed to a tuberculous environment over 2 years. No lesions of tuberculosis.

No. 435, killed April 10, 1909, after having been exposed to a tuberculous environment over 2 years. One post-pharyngeal gland twice the normal size and almost entirely tuberculous. No other lesions.

No. 431, killed March 25, 1909, after having been in a tuberculous environment almost 2 years. Mediastinal glands contained a number of minute necrotic foci which failed to show the presence of tubercle bacilli on microscopic examination and guinea-pig inoculation. Lungs contained a number of minute areas which had the appearance of healing tubercles; no bacilli found in these areas.

No. 436, killed March 29, 1909, after having been in a tuberculous environment almost 2 years. No lesions of tuberculosis.

No. 438, killed April 10, 1909, after having been in a tuberculous environment over 2 years. No lesions of tuberculosis.

No. 441, killed April 9, 1909, after having been in a tuberculous environment over 2 years. Autopsy showed minute lesions in the azygos lobe of lung and a small focus 3 mm. in diameter in the right prescapular gland. No tubercle bacilli found in the lung lesions. The prescapular lesions showed tubercle bacilli on microscopic examination and guinea-pig inoculation.

No. 439, killed March 29, 1909, after having been exposed about 16 months to a tuberculous environment. Tubercles in the posterior mediastinal glands, which on guinea-pig inoculation caused generalized tuberculosis.

#### TEST ACCORDING TO PEARSON'S METHOD.

On June 2, 1906, the first 9 calves, and on June 12, the tenth calf in the following list were given each an intrajugular injection of 3 c. c. of a suspension of tubercle bacilli of the human type. The tubercle culture used to make the suspension was "Case 32," and each cubic centimeter of the suspension was equal to 0.0013 gram of tubercle bacilli.

Heifer calf No. 412, about 4 months old.  
Heifer calf No. 413, about 3 months old.  
Bull calf No. 425, about 2½ to 3 months old.  
Bull calf No. 426, about 2½ to 3 months old.  
Bull calf No. 428, about 2½ to 3 months old.  
Heifer calf No. 430, about 2½ to 3 months old.  
Bull calf No. 433, about 2½ to 3 months old.  
Bull calf No. 440, about 2½ to 3 months old.  
Heifer calf No. 442, about 2½ to 3 months old.  
Heifer calf No. 445, about 3 months old.

The ages of the calves as given above refer to the date on which the injections were made.

On July 18, 1906, the 10 calves were given a second intrajugular injection of a suspension of tubercle bacilli in all respects relative to dose and strength similar to the first injection.

On September 11, 1906, the calves received a third intrajugular injection of a suspension of tubercle bacilli. The third dose, like the other two, was 3 c. c., but the suspension was stronger, 1 c. c. being equal to 0.002 gram of tubercle bacilli.

With the exception of some elevation in temperature, most marked after the third injection, the calves showed no pronounced symptoms as the result of the injections.

Of the 10 calves, 1 (No. 445) was killed and examined post-mortem without being exposed to tuberculosis, to determine what lesions, if any, had been caused by the three injections of tubercle bacilli. The remaining 9 calves were exposed to a tuberculous environment, as follows:

- Nos. 412 and 425, October 30, 1906.
- Nos. 413 and 426, February 21, 1907.
- Nos. 428 and 430, April 25, 1907.
- No. 440, November 8, 1907.

Control animals were added at the time of each exposure. The character of the tuberculous environment has already been described.

The dates on which the 10 calves were killed and a short memorandum of the lesions found on autopsy follow:

No. 445, treated but not exposed, reacted to tuberculin October 17, 1907 (about 13 months after last bovo-vaccination injection), killed November 7, 1907. No lesions of disease.

No. 412, killed April 28, 1908, after having been exposed to a tuberculous environment about 18 months. No lesions of disease.

No. 425, died December 18, 1906, after having been exposed to a tuberculous environment about 6 weeks. Cause of death, inflammation of the intestines. No lesions of tuberculosis.

No. 418, killed August 9, 1909, after having been exposed to a tuberculous environment over 2 years. No lesions of disease.

No. 426, killed April 1, 1909, after having been in a tuberculous environment over 2 years. Lungs were found to contain a few very minute tubercles.

No. 428, killed March 29, 1909, after having been in a tuberculous environment nearly 2 years. No lesions of tuberculosis.

No. 430, killed March 22, 1909, after having been in a tuberculous environment nearly 2 years. The pleura and mediastinal glands showed minute lesions closely resembling tuberculosis, but no tubercle bacilli could be found in these lesions by microscopic examination or guinea-pig inoculation.

No. 433, killed July 16, 1909, after having been exposed to a tuberculous environment about 2 years. No lesions of tuberculosis.

No. 442, killed July 16, 1909, after having been exposed to a tuberculous environment about 18 months. No lesions of tuberculosis.

No. 440, killed April 10, 1909, after having been exposed to a tuberculous environment nearly 18 months. No lesions of tuberculosis.

#### VACCINATION BY SUBCUTANEOUS INJECTIONS.

##### INJECTIONS INTO THE END OF THE TAIL.

As the earlier investigations of the Bureau of Animal Industry on the subject of protective inoculations against tuberculosis (see Bulletin 52, Part III) had given results indicating that the degree of immunity conferred by the injection of living tubercle bacilli into the bodies of cattle depended rather upon the virulence of the injected bacilli than upon the method of injection or the number of injections, a series of tests was made relative to the effects from injecting cattle with quite virulent tubercle bacilli into a portion of the body (the end of the tail) from which the infection, with its strong tendency to become localized, would have to move some distance before it could reach its favorite location in the body. The end of the tail also offered the advantage that the character and process of the inoculation disease could be watched and that treatment, surgical if necessary, could be applied.

On June 20, 1906, the following 4 calves received each a subcutaneous injection, immediately above the brush at the end of the tail, of 3 c. c. of a suspension of bovine tubercle bacilli. The tubercle

culture used was "Bovine III," and each 3 c. c. of the suspension was equal to 0.01 gram of tubercle bacilli.

Heifer calf No. 447, about 3 months old.

Heifer calf No. 448, about 3 months old.

Bull calf No. 450, about 3 months old.

Bull calf No. 451, about 2½ months old.

The tuberculous disease caused in the tails of the animals varied considerably. In one case, No. 447, it was necessary to amputate the tail. The four animals were exposed to a tuberculous environment beginning some time after the protective injections had been made. Later on, when they were killed and examined post-mortem, all with the exception of No. 450 were found to have tuberculous lesions directly traceable to the tubercle bacilli injected into the ends of their tails, proving definitely that the strain of tubercle bacillus used was too virulent for the injection of calves in any manner.

Calf No. 450, after remaining in a tuberculous environment for several years, was found on autopsy to be in excellent condition and entirely free from lesions of tuberculosis.

On June 20, 1906, the following 5 calves received each a subcutaneous injection, immediately above the brush at the end of the tail, of 3 c. c. of a suspension of a virulent human tubercle bacilli. The tubercle culture used was "Boy V," and each cubic centimeter of the suspension represented 0.01 gram of tubercle bacilli.

Heifer calf No. 449, about 3½ months old.

Bull calf No. 452, about 2½ months old.

Bull calf No. 453, about 2½ months old.

Bull calf No. 454, about 3 months old.

Heifer calf No. 455, about 3 months old.

With the exception of a slight swelling at the seat of injection, which gradually subsided, the treatment received by the calves caused no visible lesions.

Some time after the protective injections were made the calves were exposed to a tuberculous environment, and later on they were killed and examined post-mortem.

Calves 449 and 453 showed no lesions of tuberculosis as a result of either the protective injections or the exposure.

Calves 452 and 454 each showed a small tuberculous abscess at the seat of inoculation in the tail and small tuberculous foci in the coccygeal lymph glands (the lymph glands located near the root of the tail), and no lesions as a result of the exposure to tuberculosis after the protective injections had been made.

Calf 455 was found on autopsy to have tuberculous lesions in the coccygeal and pharyngeal glands, or, in other words, to have contracted tuberculosis both from the protective injection and the subsequent exposure.

All the tuberculous lesions found were small, but calves 452, 454, and 455 show conclusively that the strain of tubercle bacillus injected was too virulent to be used for immunizing purposes. Calves 449, 452, 453, and 454 indicate that even a subcutaneous injection of tubercle bacilli can protect against subsequent exposure to a tuberculous environment, and No. 455 shows that a tuberculous process induced by inoculation does not necessarily, in all instances, protect against fresh infection from without, and this is one of the most important facts with which we have to deal in the question of bovo-vaccination.

On November 14, 1906, the following cattle received each a subcutaneous injection, immediately above the brush at the end of the tail, of 3 c. c. of a suspension of bovine tubercle bacilli. The tubercle culture used was "Bovine III," and each cubic centimeter of suspension was equal to 0.01 gram of tubercle bacilli.

Heifer No. 406, about 18 months old.

Heifer No. 386, about 16 months old.

Cow No. 336, about 3 years old.

Cow No. 215, about 6 years old.

One of the above animals (cow No. 336) died about four months after injection without additional exposure to tuberculosis. The cause of death was inflammation of the intestines, but she showed a well-marked tuberculous lesion in her tail at the seat of injection and tuberculous lesions in four mediastinal glands. All other parts of her body were free from tuberculosis.

Heifers 406 and 386 and cow 215 were killed after having been exposed to a tuberculous environment for quite a long time, and showed tuberculous lesions which might have been caused in part by the exposure, and which were certainly caused in part by the injection of tubercle bacilli.

#### INJECTION UNDER THE SKIN OF THE NECK.

On June 21, 1906, the following 5 calves were each injected with 3 c. c. of a suspension of virulent human tubercle bacilli. The dose in each case was divided into two parts, and one part was introduced under the skin on the right side of the neck and the other part under the skin on the left side of the neck. Each 3 c. c. of the suspension injected represented 0.01 gram of tubercle bacilli.

Bull calf No. 456, about 2½ months old.

Bull calf No. 457, about 3 months old.

Bull calf No. 458, about 2½ months old.

Heifer calf No. 459, about 2½ months old.

Heifer calf No. 462, about 2½ months old.

Calves 458 and 462 died in about two months as a result of tuberculosis caused by the injection of tubercle bacilli. The remaining

three animals were exposed to a tuberculous environment for some time and were subsequently killed and examined post-mortem. All showed extensive lesions of tuberculosis, no doubt almost entirely due to the injection of tubercle bacilli, showing that either this method of treatment was too severe or that the culture of tubercle bacilli used was too virulent.

In addition to the foregoing subcutaneous injection, 10 calves, Nos. 446, 460, 463, 464, 465, 466, 469, 470, 473, and 474, were each given two successive injections of virulent tubercle bacilli, with an interval between the two injections. These calves all contracted tuberculosis from the treatment received, and simply emphasized that subcutaneous injections, no matter what part of the body is used as the seat of injection, are dangerous when the bacilli injected have a true virulence for cattle. In a later and fuller report on this work it is expected that the lesions caused by the various injections may be given and discussed in detail.

Among the subcutaneous injections only those made into the ends of the tails of calves 449, 452, 453, 454, and 455 with virulent human cultures hold out any encouragement that a subcutaneous method of protective inoculations against tuberculosis can be developed. The results with these animals, however, are quite encouraging and indicate the advisability of making tests of successive injections into the tails of cattle, beginning with fairly virulent cultures.

#### CHECKS OR CONTROL ANIMALS.

The following 11 untreated cattle, which were similar in age, size, and condition to those given intravenous protective treatment according to the methods of Pearson and Von Behring, were exposed for the same length of time to the same tuberculous environment to which the treated or protected animals were exposed. For the sake of brevity only a sufficient account of these animals will be given now to show that the environment contained the necessary amount of virulent, infectious material to cause tuberculosis of most cattle exposed in it.

Heifer No. 471 contracted tuberculosis of the mediastinal and mesenteric glands.

Heifer No. 472 contracted tuberculosis of one post-pharyngeal gland.

Heifer No. 475 contracted tuberculosis of the mediastinal glands.

Heifer No. 526 contracted tuberculosis of the mediastinal glands.

Steer No. 530 contracted tuberculosis of the lung and mediastinal glands.

Steer No. 531 remained healthy.

Heifer No. 539 contracted tuberculosis of one post-pharyngeal gland.

Heifer No. 540 contracted generalized tuberculosis.

Heifer No. 570 contracted tuberculosis of the mediastinal glands.

Bull No. 573 contracted tuberculosis of the lung and mediastinal glands.

Cow No. 579 remained healthy.

Of course it is to be understood that these cattle were carefully tested with tuberculin and found to be free from tuberculosis before they were exposed as control animals in the experiment. It is desired to avoid all detail not to some extent essential for a general statement in the present report.

In addition to the check or control cattle specially related to the tests of the Pearson and Von Behring methods of bovo-vaccination, three other check animals were also introduced into the same tuberculous environment, which was in all instances used as the means to test the amount of immunity acquired by the treated animals in these series of experiments. The three additional animals were Nos. 477, 484, and 549, and all three contracted tuberculosis as the result of the exposure.

There was thus a total of 14 checks or controls, of which 12 became infected with tuberculosis, thus showing that the character of the exposure to which the treated cattle were subjected was quite severe. The time at which the various control animals were introduced into the tuberculous environment and the length of time they were permitted to remain in it were carefully planned in connection with the exposure of the treated animals, so that any failure among the latter to become infected could be properly valued through a comparison of the treated with the control animals.

One statement which has an important bearing on the intravenous methods of producing resistance against tuberculosis must be added here. All our cattle treated with intravenous injections of tubercle bacilli showed, on careful post-mortem examination, some pulmonary lesions, such as thickening of the pulmonary connective tissue, adhesions between the lobes of the lungs and of the lungs to the chest wall and diaphragm, to be sure only very slight in most instances, but which could be accounted for in no other way than as remnants of the disturbance caused by the injected bacilli. This alone constitutes a condition which should receive further attention before a system of bovo-vaccination requiring the intravenous injection of living cultures of tubercle bacilli is practiced on a wholesale scale, and, in conjunction with what we know about the retention of tubercle bacilli in the tissues after injection and the uncertainty about the manner and state in which they leave the body, should teach us to proceed cautiously in the adoption of protective methods, notwithstanding the fact that the results obtained prove conclusively that actual, strong resistance to tuberculosis can be established by using them.

## COMPARISON OF THE FOREGOING METHODS.

From the brief accounts that have been given we see that 3 of the 9 cattle treated according to the method of Von Behring and afterwards exposed contracted tuberculosis, that 1 of the 9 cattle treated by the method of Pearson and afterwards exposed contracted tuberculosis, and that of 14 checks or untreated control animals 12 became infected on exposure.

Of the 8 animals that received injections of bovine tubercle culture subcutaneously into the ends of their tails (4 old and 4 young animals), all but 1 young animal contracted disease as the result of the injections. The one that escaped disease from the injection also resisted tuberculosis on exposure.

Of the 5 cattle that received human culture injections into the ends of their tails, 2 became immune without inoculation disease, 2 were immune but had slight inoculation disease, and 1 had both inoculation and exposure disease.

The injection of tubercle cultures under the skin of the neck of animals caused them all to contract tuberculosis.

The several methods may be compared as follows:

Von Behring cattle, 66½ per cent successfully protected for a period approximating 2 years.

Pearson cattle, 88½ per cent successfully protected for a period approximating 2 years.

Bovine cultures, tail, protected 12½ per cent.

Human cultures, tail, protected 40 per cent.<sup>1</sup>

Injections under skin protected 0 per cent.

The above percentages can best be valued by comparing them with the check or control animals, among 14 of which only 2, or 14½ per cent, escaped.

If the only question to be considered in connection with bovovaccination was the protection of cattle against tuberculosis, the foregoing results would give us excellent reasons to be very cheerful. Lately, however, studies made by various investigators on the elimination of tubercle bacilli, after injection, from the bodies of animals teach us to be very careful about adopting methods of immunization for purely economic purposes that may be dangerous for those who afterwards use the products of the treated animals.

The work of the Bureau of Animal Industry in the past has also demonstrated that tubercle bacilli injected into the circulation or under the skin of cattle may remain incorporated in their tissues for long periods of time, with only a gradual or very slow loss of virulence. These are important factors that must not discourage further work to build on the knowledge we have gained, but which must be

<sup>1</sup> It should be borne in mind that the human cultures injected in the tail actually protected 80 per cent of the cattle treated against the infection in the environment to which they were exposed.

kept in mind and be permitted to have their due influence on our subsequent investigations.

Very careful autopsies of cattle treated by intravenous inoculations of tubercle bacilli according to the methods of Von Behring and Pearson show that the more or less attenuated tubercle bacilli that engender immunity against tuberculosis rarely leave the treated subjects wholly free from lesions that can be accounted for in any other way than as due to the pathogenic activity within the animal's body of the injected bacilli.

#### TEST OF THE HEYMANNS METHOD OF BOVO-VACCINATION.

A protective treatment for cattle against tuberculosis, named the Heymanns method after its inventor, has received considerable attention during the last few years. The technique of this method was demonstrated in America by Prof. Heymanns himself during the meeting of the International Congress on Tuberculosis at Washington in 1908, and the efficiency of the method has been carefully tested by the Bureau of Animal Industry with cattle and hogs.

Heymanns's method, briefly, is the introduction of virulent bovine tubercle bacilli, enveloped in a closed sack of vegetable fiber, which in turn is inclosed in a gelatin capsule, under the skin of the animal to be protected. The supposition is that the vegetable sack will confine the tubercle bacilli at the seat of inoculation and that the treated animal will be immunized by protective fluids that form within the closed vegetable sack and pass outward from it, into the animal's system generally, by an osmotic process. The closed sack of vegetable material in combination with the gelatin capsule is commonly known as the Heymanns capsule.

In the fall of 1908, 12 cattle and 10 hogs were inoculated with Heymanns's capsules. The animals were divided into three groups, and one group of hogs and cattle was exposed immediately after treatment to a tuberculous environment, a second about 2 months later, and a third about 2 months after the second. With each group of hogs a similar number of untreated hogs were exposed as checks. As the cattle and hogs were exposed in the same tuberculous environment in which the degree of immunity acquired by all the other bovo-vaccinated animals discussed in this paper was tested, no checks were really necessary for the Heymanns treated cattle. The checks on the other experiments were serviceable for this one also, but, nevertheless, 4 additional cattle as special checks on the value of the Heymanns method were added to the already large number used to prove the character of the tuberculous environment to which exposure was made.

Among the Heymanns treated hogs 1 contracted generalized tuberculosis from the treatment and 1 died prematurely as the result of

an injury. When the remaining 8 hogs with their 10 checks were killed, after an exposure to natural infection varying from 8 months to a year, all the principals and checks were found to be affected with tuberculosis, not one of either lot having escaped the disease; and the lesions in the treated animals were in no respect different from those found in the checks. Hence it is very clear that Heymanns's method is absolutely worthless for hogs.

Among the 12 cattle treated 1 died prematurely, and the remaining 11, when they were killed and examined post-mortem, all showed lesions of tuberculosis, and the lesions were very similar to the tuberculous lesions found in the 4 cattle that served as special checks on the Heymanns capsule cattle. One of the treated animals showed tuberculosis directly traceable to the capsule with which it was inoculated for protection. Hence, as with the hogs, the only conclusion that can be drawn with the cattle is that Heymanns's capsule method of bovo-vaccination is inefficient.

In order to test the claims made by Prof. Heymanns, that the vegetable sack in which he incloses the tubercle bacilli used in his method of bovo-vaccination would not permit the passage of bacteria, a number of sheep were inoculated with anthrax bacilli inclosed in Heymanns's capsules. The sheep rapidly contracted and succumbed to anthrax, and the anthrax bacilli (which are larger, of course, than tubercle bacilli) were proven to have escaped through the walls of the capsules and to have gotten into the blood circulation. Blood examined from the tips of the ears of the sheep showed numerous anthrax bacilli. We may conclude from this that the fact that tubercle bacilli introduced under the skin in Heymanns's capsules are frequently restrained at the point of inoculation depends upon other conditions than the inability of the germs to pass through the walls of the vegetable sack in which they are enveloped.

#### BLOOD TRANSFUSIONS.

It may be of interest to add a short note that blood transfusions from highly immunized cattle into tuberculous cattle for curative purposes and into healthy cattle in order to make them resistant to tuberculosis have been tested on a small scale. The idea that such transfusions may give good results originated with Dr. George W. Crile, of Cleveland, Ohio, who personally made the various transfusions required in the experiment.

The blood for the transfusions was supplied by cattle that had been immunized by the methods of Pearson and Von Behring and that had resisted infection after a long-continued exposure to the tuberculous environment previously described, in which about 86 per cent of all exposed, untreated, or check cattle contracted tuberculosis.

As far as the very small number of cattle used justifies drawing conclusions, the blood-transfusion experiments gave wholly negative results and hold out no encouragement as being a means by which tuberculosis can be treated or the resistance to infection strengthened. The treated animals may have been too far advanced in the disease to derive benefit from any form of treatment, but the animals which received blood to immunize them apparently were as susceptible to infection as those that had received no treatment.

#### GENERAL CONCLUSIONS.

The only conclusion to which we are entitled from this work and from careful study of the writings of others on the subject of protective inoculation against tuberculosis may be stated as follows: Though results have been obtained which are very encouraging to the investigator and which prompt him to strive onward with renewed vigor and hope, no system of bovo-vaccination has reached a stage at the present time that justifies its use in common practice.

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